

**REVIEW****The expanding world of tissue-resident macrophages***Stephen J. Jenkins<sup>1</sup> and Judith E. Allen<sup>2</sup>*<sup>1</sup> Centre for Inflammation Research, Queens Medical Research Institute, University of Edinburgh, Edinburgh, UK<sup>2</sup> Lydia Becker Institute of Immunology & Inflammation, Wellcome Centre for Cell Matrix Research, School of Biological Sciences, University of Manchester, Manchester, UK**50<sup>th</sup>**  
anniversary  
**REVIEW series**

The term ‘macrophage’ encompasses tissue cells that typically share dependence on the same transcriptional regulatory pathways (e.g. the transcription factor PU.1) and growth factors (e.g. CSF1/IL-34). They share a core set of functions that largely arise from a uniquely high phagocytic capacity manifest in their ability to clear dying cells, pathogens and scavenge damaged, toxic or modified host molecules. However, macrophages demonstrate a remarkable degree of tissue-specific functionality and have diverse origins that vary by tissue site and inflammation status. With our understanding of this diversity has come an appreciation of the longevity and replicative capacity of tissue-resident macrophages and thus the realisation that macrophages may persist through tissue perturbations and inflammatory events with important consequences for cell function. Here, we discuss our current understanding of the parameters that regulate macrophage survival and function, focusing on the relative importance of the tissue environment versus cell-intrinsic factors, such as origin, how long a cell has been resident within a tissue and prior history of activation. Thus, we reconsider the view of macrophages as wholly plastic cells and raise many unanswered questions about the relative importance of cell life-history versus environment in macrophage programming and function.

**Keywords:** Cellular immunology • Dendritic cells • Infection • Macrophages • Monocytes

**Introduction**

The study of macrophages has been one of the most dynamic and fast-moving fields in biology in the past 30 years. In the 1990s, macrophages were viewed primarily as antimicrobial phagocytes and were the poor cousins to the more popular DCs in the myeloid cell family. This began to change with the seminal work of Siamon Gordon, illustrating that macrophages responded very differently to distinct immune signals [1]. For the next 10–15 years, our focus was on macrophage activation states driven by local immune signals. This led to a far greater understanding of the role of macrophages in tissue repair and nonimmune tissue homeostatic functions [2]. Together, these discoveries led to the accepted

view of macrophages as remarkably plastic and flexible cells that can change their physiology in response to environmental cues [3].

In the 2010s, a new revolution occurred in the macrophage field with the discovery that many if not most tissue-resident macrophages (TRMs) had embryonic origins and were maintained by local self-renewal rather than recruited from the blood [4–6]. This discovery was intimately tied to a new understanding that macrophage proliferation plays a key role in maintenance or expansion of specific macrophage populations. Our own contribution to this revolution was the finding that in the context of the type 2 cytokines, IL-4 and IL-13, macrophage numbers can increase through local proliferation as an alternative strategy to blood cell recruitment during infection or injury [7, 8].

The identification of different origins raised the question of whether ontogeny (intrinsic signals) or the microenvironment in which the cell resides (extrinsic signals) are the major

**Correspondence:** Dr. Stephen J. Jenkins and Prof. Judith E. Allen  
e-mail: stephen.jenkins@ed.ac.uk; judi.allen@manchester.ac.uk

determinant of cellular identity. In an insightful review, the ontogeny, local environment, inflammation status, and time spent in tissues are described as the factors that determine macrophage phenotype [5]. It is clear, we have moved far beyond the original descriptions of polarised macrophage activation states. This new understanding brings enormous complexity to the macrophage field that will require new insight to define the impact of all these permutations on function. This review aims to highlight many of the questions that remain unanswered in the face of our expanding view of macrophage life history.

## Tissue residency, density, and proliferation

Every tissue in the body has long-lived TRM populations, integral to the health of that tissue, which can function independently of blood-monocyte recruitment [5]. The level of this autonomy, however, varies dramatically between tissue sites in that some remain entirely independent of BM contribution while other TRM populations have high rates of replenishment from BM precursors or low/intermediate rates. The rate of replenishment not only differs between tissue sites but differs with age [9] and sex [10].

Tissue autonomy requires local macrophage self-renewal, a feature of TRMs regardless of origins [4]. However, a key question that arises, relevant to both the steady state and inflammatory settings is **What limits the number of TRMs?** Typically, what limits cell density is the “carrying capacity” of a tissue and is determined by multiple factors including nutrients, oxygen, physical space, and critical growth factors [11]. For most macrophages, signaling through CSF1R (the receptor for CSF-1 and IL-34) is a major determinant of survival and proliferation. The fact that cell surface and secreted forms of CSF1 appear to have unique roles in macrophage maintenance led to the hypothesis that defined niches or territories exist, controlled by local production of these growth factors [12]. Thus, the profile of CSF1 expressing cells may determine spatial distribution of certain TRM populations. Zhou et al. [11] propose that macrophage proliferation is not limited by physical constraints such as contact inhibition to the same degree as stromal cells. They model a two-cell circuit, in which cell contact between fibroblasts producing CSF1 and macrophages producing PDGF is beneficial due to optimal growth factor exchange. In this scenario macrophage numbers are capped at a 1:1 ratio by the number of fibroblasts [11]. In support of this model, hepatic stellate cells, the main local producers of CSF1 in the liver, are intimately associated with roughly equal numbers of Kupffer cells (KC), suggesting the spatial distribution of hepatic stellate cells controls the individual territories of KC [13].

The interdependence between macrophages and stromal CSF1-producing cells highlights an important feature of many TRMs; they can be remarkably immobile. For example, both KC [13] and alveolar macrophages [14] form sessile and stable interactions with structural cells. Similarly, TRMs in the peritoneal wall are stationary and evenly distributed throughout the tissue, with tissue-protective functions performed by multiple spread-

ing pseudopods. When pseudopods are insufficient to deal with an insult, the TRMs recruit granulocytes and monocytes [15]. Macrophage immobility is nicely illustrated in the biology of tattoos. Dermal macrophages are the primary store of long-term tattoo color and the pigment remains in place due to cycles of capture-release-recapture [16]. However, growth factor availability from neighboring cells may not always limit TRM number or location as macrophages can be autocrine for CSF1 [17, 18]. Alveolar macrophages in the asbestos-induced fibrotic niche produce PDGF that supports CSF1-producing fibroblasts, but these macrophages, in both humans and mice, can themselves produce CSF1 [19]. Autocrine CSF1 may, therefore, allow fibrosis to spread. We, therefore, need to know: **Is autocrine CSF1 a uniquely pathologic process?** and what determine the limits, if any, to macrophage expansion in disease settings. Our own data show that constraints on macrophage numbers normally mediated by CSF1 availability can be overcome by IL-4 during nematode infection [7]. Whether the ability of IL-4 to override CSF1-dependence applies in other inflammatory settings, or whether other cytokines function similarly, remains unclear.

Numerous autonomous TRM populations, including KC [20, 21], Langerhans cells [22], peritoneal [23, 24], alveolar [9, 25–27], and meningeal macrophages [28], can be replenished from the BM during local inflammation. **What then controls autonomy of TRMs?** Most data suggest that engraftment of recruited inflammatory macrophages requires loss of TRMs because replenishment correlates with the degree of resident cell disappearance that follows an inflammatory insult [9, 24] (aka the macrophage disappearance reaction). Tran et al. [21] in a model of chronic nonalcoholic steatosis, provide formal evidence that progressive death of established KC is needed for the development of monocyte-derived KC. Experimental depletion systems have consistently demonstrated that extensive loss of TRMs provides the space for monocytes to replenish and generates the signals for monocytes to be recruited to tissues areas where they would not normally migrate [13, 29]. Notably, following partial depletion, repopulation by monocytes appears relatively inefficient compared with proliferation of residual resident cells [13, 24, 29, 30]. Why this is remains unclear, but TRMs may be optimally programmed to efficiently use locally available fuels [31] and compete for growth factors such as CSF1 [32]. This would explain why in a setting of limited TRM loss (i.e. death of individuals cells) replenishment would most likely be via proliferation of established macrophages.

If macrophages have long-term self-renewal capacity, **why do monocytes engraft in the steady-state?** One hypothesis is that low-grade inflammation provides monocyte access. Is there minor continual disappearance reaction in some tissues, triggered by microbial products or mechanical stress? Fate mapping studies suggest that replacement of embryonically derived TRMs with monocytes reaches a plateau by 12 weeks of age in many of these tissues [9]. This would suggest an alternative hypothesis in which tissue growth or development is the trigger for monocyte engraftment, consistent with the mouse uterus during pregnancy, in which monocyte recruitment and macrophage proliferation

both play major roles [33]. Similar processes may occur during cancer, when the incoming monocytes engraft into the new tissue and then proliferate as the tumor grows in size [34, 35]. In addition, although tissue sites that promote stable sessile interactions between macrophages and stroma are predicted to endow resistance to displacement by monocytes [6], in the tattoo studies dermal macrophages turnover relatively rapidly from monocytes and yet remain highly stationary [16]. Hence, it is possible that some tissue sites are less permissive for TRM proliferation. Indeed, dermal and gut TRM populations are reduced in monocytopenic *Ccr2*<sup>-/-</sup> mice [36, 37] raising the question: **What is the evolutionary advantage of obligatory replenishment by monocytes?** Many intracellular pathogens preferentially infect macrophages and, thus, monocyte replenishment at sites of high risk of infection may allow continual elimination of pathogen reservoirs.

If monocytes can replace tissue-resident cells of embryonic origin, **How important is it that a macrophage can proliferate?** Methods that analyze proliferation history demonstrate that population density is maintained by self-renewal rather than simply cell longevity [10, 38–40]. In this context, the role of macrophage proliferation is to fill an empty or reduced niche and can be directly coupled to death of neighboring cells [41]. However, during certain types of inflammation proliferation can occur even when the niche is apparently full [7, 8, 42, 43]. Indeed, proliferation can lead to a dramatic increase in macrophage numbers well beyond the steady state without monocyte input, but the functional consequences of this proliferative response remain mostly obscure. Helminth infection leads to IL-4R $\alpha$ -mediated proliferative expansion in multiple tissues [44] as in tissue injury [45]. In some settings, CSF1 can itself act to expand macrophage numbers above steady-state levels [34, 35, 46]. It is challenging to establish the direct functional consequences of macrophage proliferation because CSF1 or IL-4R $\alpha$  inhibition have effects well beyond macrophage proliferation. For example, macrophages in the atherosclerotic lesion can increase in number via proliferation [47], and there is compelling evidence that TRMs which expand through proliferation, promote tumor progression during pancreatic cancer [48]. However, in the absence of tools to specifically block macrophage proliferation, it is not possible to determine whether proliferation is a significant contributing factor to disease. To address this question, we need to know **what specifically regulates macrophage proliferation?** Although closely tied to the metabolic state of the cell [49, 50], our current understanding of TRM proliferation, both mechanistically and functionally, still remains limited (see Fig. 1).

Evidence that established and monocyte-derived resident macrophages can differ functionally during disease will be discussed in the next section but provides a key argument as to why proliferation may matter. Notably, in the uninflamed peritoneal cavity [10], the lung following influenza infection [26] and in the liver following diet-induced inflammation [21] proliferation is greater in TRMs of recent monocyte origin, a characteristic which is reflected in the hyperproliferative state of monocyte-derived KC repopulating an empty niche [13]. This hyperproliferative state appears to allow immature resident cells to outcompete

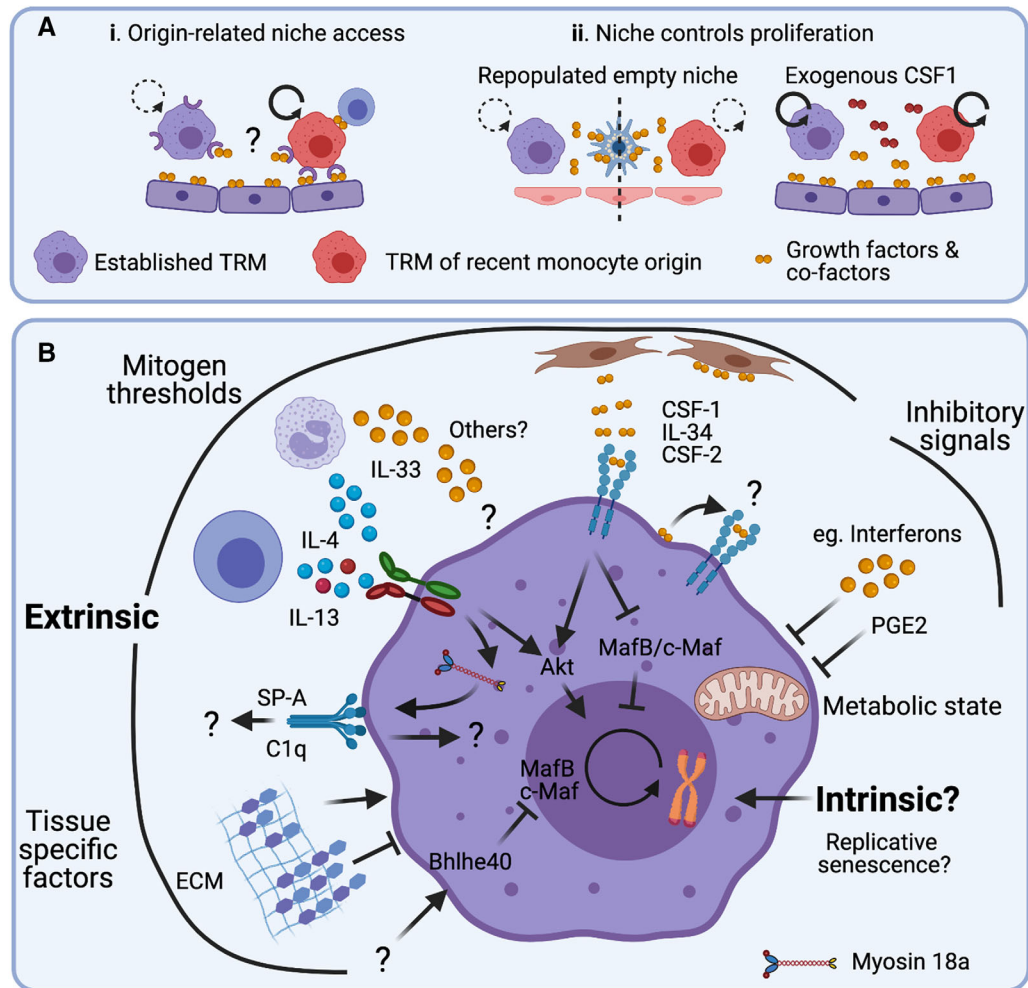
established resident cells in lung [26] and more rapidly repopulate an experimentally depleted peritoneal niche [24], yet under steady-state conditions established TRMs are maintained [24]. **What protects older resident macrophages from competition by the more-proliferative newcomers?** Proliferation is presumably coupled with compensatory differences in survival and death but to understand these dynamics, we need better tools to measure rates of survival and death. As the difference in proliferation between immature resident cells and established resident cells can be overcome by delivery of excess exogenous CSF1 [10], the ability to access endogenous growth factors rather than cell-intrinsic mechanisms, such as exhaustion or senescence, may explain these differences. However, we also still do not know whether individual macrophage clones have an intrinsic proliferative limit. If macrophage proliferation eventually results in telomere loss and senescence, this will affect how they change during aging or following chronic or repetitive inflammatory disease. The balance between “new” and “old” in a resident pool, thus, has particular relevance to aging and raises the question, addressed by Soucie et al. [51]: **How stem-like are TRMs in their self-renewal capacity?**

## Cellular identity

The scale of macrophage heterogeneity is vast and new technologies have accelerated discovery of unique populations with critical tissue-specific functions [5]. These discoveries have revealed a multilayered system (see Fig. 2), that determines the ultimate identity, activation state, and function of a TRM. Starting with the lineage-determining transcription factor PU. 1 (layer 1) [52], these layers by and large require continual exposure to local conditioning/activating signals, but their effect may be modified by the prior life history of a macrophage, including ontogeny, time of tissue residence, and history of activation/tissue residence. It is beyond the scope of this review to cover all of these layers in depth, but we hope to highlight key open questions regarding the intrinsic and extrinsic features that determine cellular identity and, thus, macrophage function.

### Tissue-resident cellular identity (the second layer)

The instructive role of the tissue environment in establishing TRM phenotype is now well established. Elegant mouse studies employing systems in which TRMs are either experimentally depleted or tissues lacking TRMs are engrafted with different precursors have shown that monocyte-derived cells can differentiate and largely acquire the self-replicating and tissue-specific functional characteristics of the cells they replace [29, 53–56]. Such programming is sufficient to prevent alveolar proteinosis that would otherwise occur in the absence of alveolar macrophages [54, 57] and, in the case of KC to protect against intravascular infection and regulate the response to local tissue injury [55, 56] and for microglia, to support normal cognitive function [58].

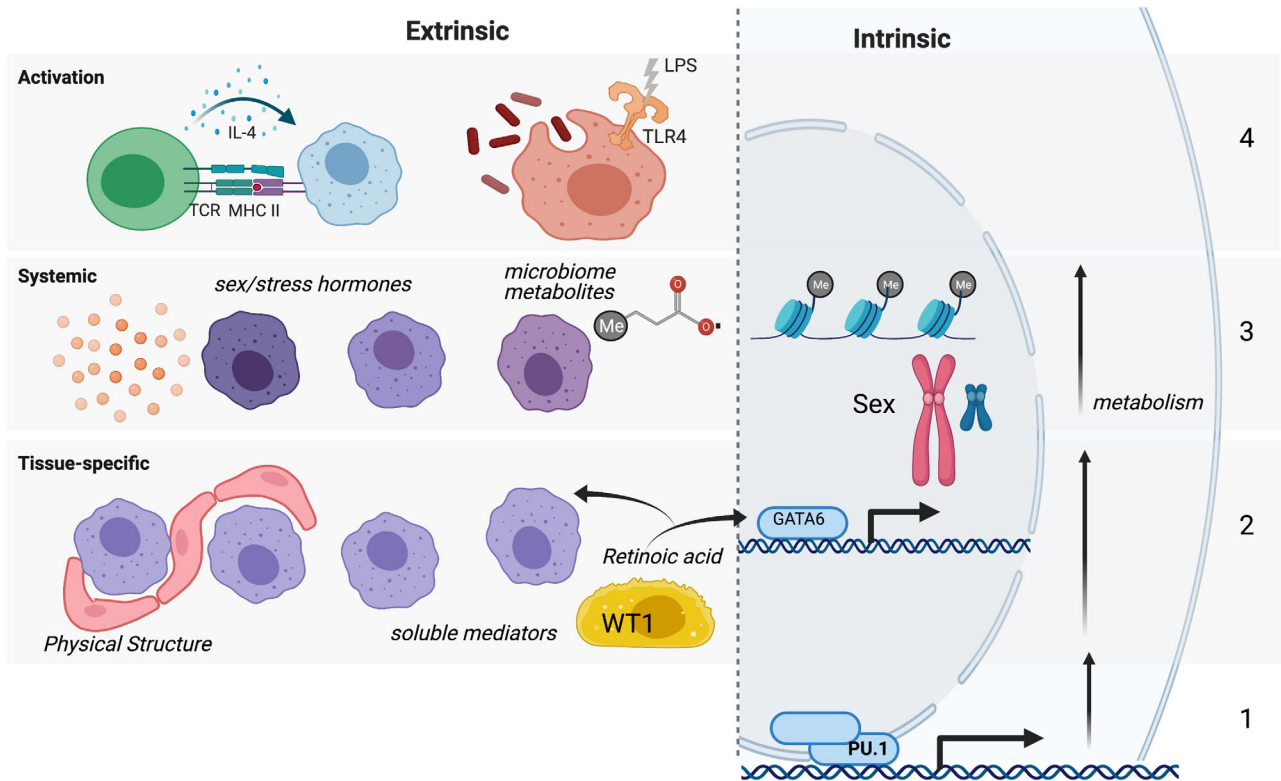


**Figure 1.** What regulates macrophage proliferation? (A) Tissue-resident macrophages (TRMs) of recent BM origin exhibit heightened proliferative activity in some tissues and pathologies (i). However, depletion and repopulation studies demonstrate equal proliferative activity regardless of origin (ii) suggesting proliferation is controlled by the niche. Also, both established and recently BM-derived TRM undergo extensive proliferation on treatment with excess growth factor (ii). Thus, origin-related differences (i) are likely explained by access to distinct sources of growth factors (GF), cofactors, or other niche signals. (B) The primary regulator of proliferation in many settings is exposure to sufficient levels of the GFs (i.e. CSF1, IL-34, and/or CSF2). Whether a GF threshold is achieved will be determined by local and in some cases systemic GF production combined with competition from neighboring TRM. Th2 cytokines (IL-4, IL-13, & IL-33) can drive proliferation independently of CSF1/CSF2 thereby overcoming innate constraints on TRM population size [7, 118]. Macrophage proliferation involves multiple signaling pathways, including activation of Akt [119, 120] and downregulation of the transcription factors MafB and cMaf, which cause cell-cycle arrest [51], and is highly dependent on metabolic state [50]. However, full knowledge of macrophage proliferation, is still limited. For, example: What is the role of autocrine CSF1? What tissue-specific co-factors beyond those already identified (C1q, SP-A via Myosin18a) [84, 121] are needed for IL-4 and IL-13-mediated proliferation? What immune or tissue signals, such as PGE2 [49] or interferons, inhibit proliferation? The transcription factor Bhlhe40 regulates cell cycle progression specifically in peritoneal macrophages [122]. Are equivalent tissue-specific transcriptional regulators of cell cycle found elsewhere? How does the composition of extracellular matrix (ECM) affect proliferation? Are TRM stem-like in proliferation capacity or do they have a replicative limit?

Substantial progress has been made in understanding the transcription factors and enhancer landscapes that regulate the specialization of resident macrophages in many tissues [5]. Headway has also been made in identifying the drivers that underlie specialization in certain tissues. For example, the transcriptional identity and survival of peritoneal TRMs is in part controlled by GATA6 expression in response to retinoic acid metabolites [59, 60] produced by mesothelial cells and fibroblastic stromal cells that express the transcription factor Wilms' Tumor 1 (WT1) [61]. Retinoic acid-independent hallmark genes of peri-

toneal macrophages are also controlled by WT1+ stromal cells but the molecular cues responsible have yet to be identified [61]. In the liver, identity of KC cells relies on the transcription factors LXRα [62] and Id3 [63]. Expression of the Notch ligand DLL4 by liver sinusoidal endothelial cells drives differentiation of monocytes to KCs by rapidly inducing LXRα expression, and increasing responsiveness to LXRα-inducing signals produced by hepatic stellate cells [13, 64]. Despite these advances and others reviewed in [6], for most tissue sites we still do not know **What signals and molecular mechanisms imprint tissue-specific functions?**





**Figure 2.** What determines TRM identity? The founding layer of TRM identity is determined largely by the transcription factor PU.1 [52]. In the unperturbed state, the next layer of identity is the tissue-specific features of TRMs that allow performance of functions essential for that organ. This cellular identity, also under transcriptional and epigenetic control [102, 106, 123], is determined by the instructive environment of the specific tissue. For example, serous cavity TRM function is determined by GATA6 expression, which occurs in response to retinoic acid (RA) metabolites [59, 60] produced by WT1+ stromal cells [61]. In other tissues, the instructive environment may include not only soluble mediators but physical properties such as extracellular matrix composition [94]. A third layer is functional alteration by systemic signals such as stress and sex hormones, microbiome-related signals, and altered host metabolism. The fourth layer is direct immune activation of macrophages, typically by other immune cells (e.g. IL-4 producing T-cells) or pathogen-derived molecules (e.g. LPS acting via TLR4), that may act locally or systemically, dependent on the nature of the insult. This fourth layer is likely to be dominant because of the evolutionary imperative to protect the host from infection or injury. Nonetheless, it is constrained by the capacity of the local cells to respond appropriately for that tissue, with the activation state of any given macrophage reflecting not only activation signals but also local tissue (layer 2) and systemic (layer 3) signals [124].

Achieving this level of resolution is critical if we are to understand what controls aberrant macrophage functions in disease.

### The role of ontogeny

The effect of ontogeny on TRM identity is most apparent in microglia. While BM precursors can repopulate an experimentally depleted microglial niche, these cells retain long-term transcriptional, epigenetic and behavioral differences, including a failure to express *Sall1*, a transcription factor central to microglial identity [65–68]. The transcriptional signature of BM-derived microglia largely overlaps with that of microglia from *Sall1*-deficient mice suggesting that differential *Sall1* expression largely determines origin-related gene expression [65, 66]. Two mechanisms could underlie these differences. As microglia originate exclusively from primitive yolk sac-derived macrophages [69], it is possible that yolk sac and BM progenitors differ intrinsically in their response to microglial niche signals [65–68]. However,

given that microglia undergo distinct maturation phases during development [70], it is also possible that the availability of extrinsic CNS signals that programme for *Sall1* expression change with development, such that the adult brain is not capable of imprinting the ability to express *Sall1* on newly recruited cells. Both mechanisms would rely on an element of cell-intrinsic regulation to maintain gene expression in microglia. Notably, microglia derived from yolk-sac progenitors engrafted directly into adult brain are transcriptionally much more akin to adult microglia than engrafted BM-derived cells [66] but never fully achieve adult microglial *Sall1* expression levels. Together, these data suggest an overriding effect of ontogeny (intrinsic) on microglial identity, influenced by dynamic changes between the embryonic and adult brain microenvironment (extrinsic). Where investigated in other tissue-resident cells, the impact of ontogeny is much less transcriptionally evident and programming is largely environment dependent [22, 24, 29, 53–55, 71] likely reflecting that uniquely in the brain TRMs remain fully yolk-sac derived for the life of the animal.

## The role of time

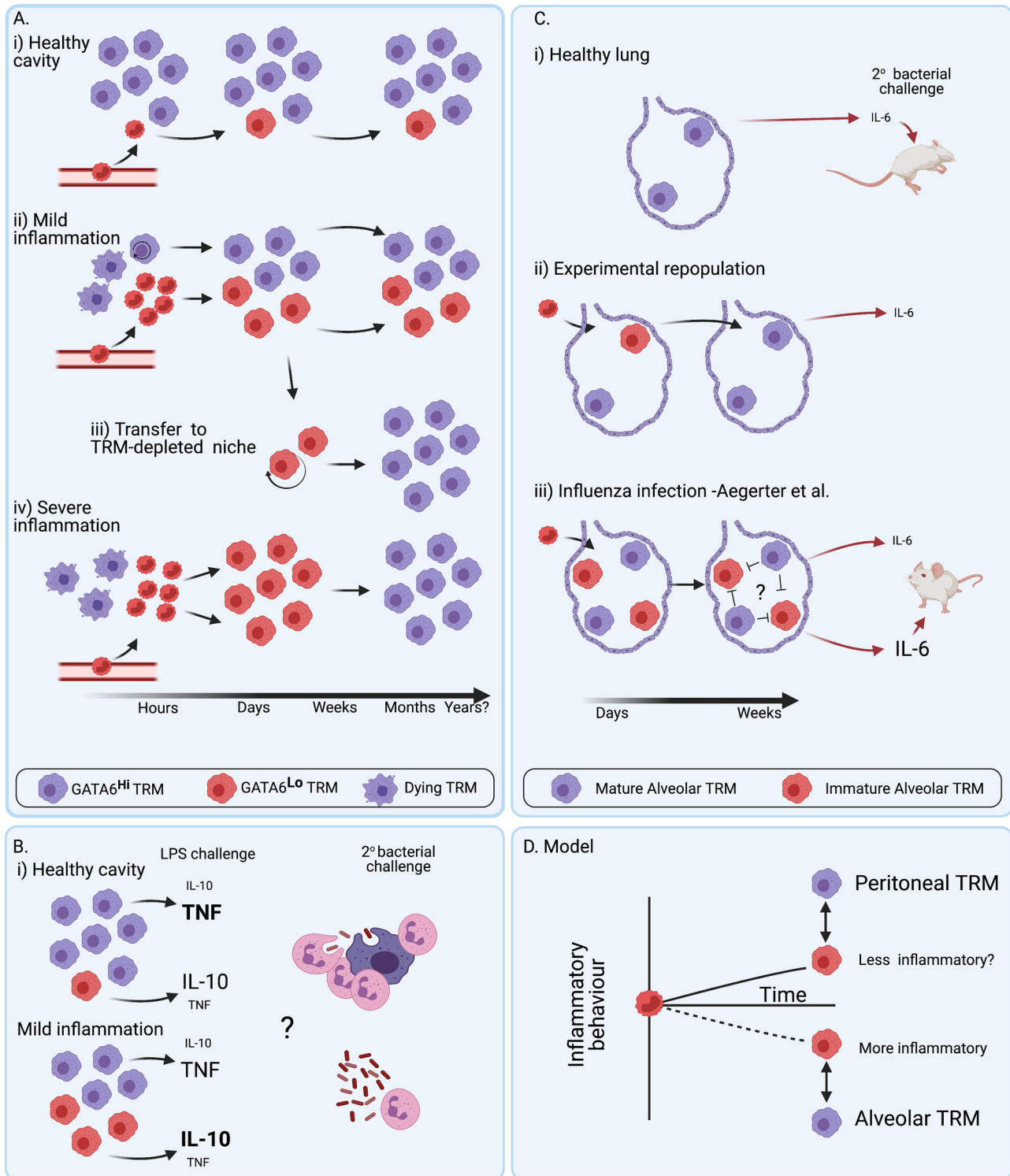
Despite the predominant role for instructive tissue signals in macrophage programming in most tissues [22, 24, 29, 53–55], expression of a few genes and proteins have been considered to be related to cell origin. The best characterization of these is the phosphatidylserine receptor Tim4, which is associated with “residency” in numerous tissue sites [5]. However, Tim4 expression appears to be related to length of time a monocyte has been resident within the tissue. This relationship with time has been most clearly demonstrated through kinetic analysis of Tim4 expression by monocyte-derived KC [29] and monocyte-derived peritoneal macrophages [24]. Expression of a number of other phagocytic receptors in various tissues is lower on recently recruited monocyte-derived macrophages relative to established resident cells (e.g. CD209 gene family, Clec12, Marco) [24, 25, 28, 29, 53–55, 71]. Where assessed (e.g. for CD209b), expression of these phagocytic receptors also appear influenced by time-of-residency [24, 71]. **What then are broader effects of time-of-residency?** Addressing this question is important because time-of-residency appears to have significant functional consequences. For example, mice exhibit more severe responses to hepatic injury and increase susceptibility to intravenous infection for a month following KC depletion [56] despite replacement by monocytes that acquire the bulk of KC identity within 3 days [13]. Similarly, impaired expression of CD209b by recently monocyte-derived macrophages is in part responsible for greater susceptibility of male mice to bacterial peritonitis [71] and is associated with defects in immune function of monocyte-derived cells that replace meningeal macrophages destroyed by viral infection [28]. In the lung, monocyte-derived alveolar macrophages recruited during influenza infection retain a transcriptional and epigenetic profile similar to that of monocytes for up to a month after infection which facilitates a period of enhanced resistance to subsequent *Streptococcal pneumonia* [26]. A major functional implication of time-of-residency (and ontogeny) is that an inflammatory event leading to substantial integration of monocytes may be akin to resetting the clock of TRM identity [5]. Alternatively, chronic inflammation may lead to generation of a spectrum of cell identities related to when during disease individual cells were recruited. Notably, monocyte-derived KC that replace dying KC during chronic liver disease have a reduced capacity to facilitate triglyceride storage [21] but determining whether time of residency is a factor will require understanding dynamics at the single-cell level.

A key unanswered question is **How are time-dependent changes regulated?** Time-of-residency expression could be controlled by a cell-intrinsic mechanism, perhaps similar to the “lactate timer” in which macrophage activation results in a metabolic switch with rising levels of intracellular lactate. The subsequent histone lactylation epigenetically regulates the duration of activation [72]. Alternatively, increased time of exposure to extrinsic signals that differ between tissues would be consistent with expression patterns of genes, such as *Vsig4*, which is time-dependent in the peritoneal cavity but rapidly expressed in the liver [13]. As discussed above for *Sall1* expression in microglia,

the extrinsic signals may change with developmental age. Critically, tissue insult has the capacity to override a time requirement. Monocytes recruited following severe peritoneal inflammation [24] or helminth infection [73] rapidly upregulate Tim4, presumably because those critical functions are needed. Whether intrinsic or extrinsic, time-dependent mechanisms would require cell-autonomous maintenance of these features in established cells.

Competition between incoming monocytes and established TRMs may be the underlying mechanism behind features that appear time dependent. Monocyte-derived cells recruited following mild peritoneal inflammation persist alongside long-term resident macrophages for at least 5 months, but exhibit differential expression of almost a fifth of genes including lower levels of the master transcriptional regulator GATA6 [24] (see Fig. 3A). In contrast, monocytes undergo far more rapid differentiation into residency in absence of competing resident cells [24]. Given that peritoneal macrophages move freely in a fluid environment [74], these data suggest established cells somehow retard transcriptional development *but not survival* of recruited cells, perhaps via competition for programming signals. Hence, at least in certain tissue types, access to the parameters that control survival (e.g. CSF1) must be separate from those that control programming (e.g. retinoic acid). Consistent with competition-inhibited maturation, under steady-state conditions, monocytes recruited to the cavity seemingly undergo the same retarded differentiation [24, 71]. The effect of competition with neighboring resident cells for programming signals is likely to be less important where macrophages form sessile and stable interactions with structural cells. This is supported by evidence that monocyte-derived and incumbent resident macrophages exhibit more than 98% transcriptional coalescence following repopulation of partially depleted lung [53] and liver [55] macrophage niches. **How and when does competition for residency signals affect macrophage behavior?** and **How does this affect subsequent susceptibility to disease** (Fig. 3B)? This question will likely be most important in tissue environments in which the environment is more fluidic such as the synovium or following inflammation and infection-mediated breakdown of tissue architecture and fluid influx.

The intersection of competition and time may also play out in the consequences of viral lung infections. Influenza infection leads to increased resistance to subsequent *Streptococcal pneumonia* infection due to the recruitment and persistence of a transcriptionally distinct population of monocyte-derived alveolar macrophages poised to make high levels of IL-6 [26] (see Fig. 3C). These monocyte-derived macrophages exhibit an epigenetic profile that resembles monocytes but lose their protective capacity within 2 months, leading the authors to propose that an epigenetic and time-sensitive “legacy” of monocyte origin underlies their protective function. Perhaps surprisingly then, experimental replacement of alveolar macrophages by monocyte-derived cells does not lead to a state similarly poised for high IL-6 production [26], suggesting additional adaptations in the inflamed lung environment are important for sustaining this transient monocyte-like state. Notably, experimental depletion of alveolar macrophages in naïve mice is followed by re-establishment of the normal



**Figure 3.** Does competition-inhibited maturation of TRM alter resistance to secondary infection? (A) Monocytes recruited to the peritoneal cavity under (i) non- and (ii) mild inflammatory conditions remain in a GATA6<sup>Lo</sup> state for months due to competition from enduring established GATA6<sup>Hi</sup> TRM. Transfer to a TRM-depleted cavity (iii) or severe peritoneal inflammation with complete loss of established TRM (iv) allows recruited macrophages to acquire a mature GATA6<sup>Hi</sup> phenotype. (B) GATA6<sup>Lo</sup> TRM recruited during (i) non- and mild (ii) inflammatory conditions produce less TNF and more IL-10 to challenge [24]. Notably, mild peritoneal inflammation leads to subsequent heightened susceptibility to bacterial peritonitis concurrent with reduced neutrophil recruitment [125, 126] but it remains to be established whether the increased frequency of GATA6<sup>Lo</sup> cells that occurs postinflammation underlies this susceptibility. (C) Imaging suggests that the noninflamed lung contains a single alveolar TRM for every three alveoli [14] but multiple macrophages per alveoli are shown here for simplicity (i). Following experimental depletion, normal macrophage numbers are restored by recruited monocytes that become transcriptionally akin to the remaining TRMs and produce equivalent IL-6 upon challenge (ii).

baseline density of cells [53] whereas influenza infection leads to an increase in total population size implying the previously distinct individual territories of alveolar macrophages may now be infringed by recruited cells. Hence, retarded differentiation of monocyte-derived alveolar macrophages following infection could be due to direct competition with established cells (see Fig. 3D). If so, it will be important to understand **why infection leads to prolonged increase in alveolar macrophage numbers compared to steady state?** Addressing this question may help resolve why a previous study [75] found that infection using the same strain of influenza resulted in increased susceptibility rather than resistance to bacterial pneumonia, since this earlier study found no evidence that infection caused a prolonged increase alveolar macrophage number.

### Systemic signals that alter identity and function (the third layer)

Long-range signals that programme TRM function include those produced by microbiota, such as metabolites like short-chain fatty acids [76, 77] and lactate [78], and microbiota-associated molecular patterns [79]. These can have overlapping effects on macrophages in multiple tissues, for example, regulating the tissue localization of microglia [76] and KC [79]. Sex also significantly affects TRM identity and function but this appears tissue-specific [71, 80]. **How does sex determine macrophage function?** Effects of sex could occur through systemic or tissue-specific regulation of hormones (extrinsic), regulation by sex-chromosome complement (intrinsic), or indirect pathways such as regulation of microbiota [81]. Notably, female microglia retain a more effective ability to protect against ischemic stroke following adoptive transfer into male brains [80] and microbiota influence microglial transcriptional identity in a sex-specific manner [82]. It is likely that integration of systemic signals with tissue-specific or time/competition-related signals, as discussed above, combine to determine the ultimate identity of TRMs. These studies highlight the complexity of defining TRM identity or function based on key transcription factors alone.

### Activation (the fourth layer)

A large body of literature on macrophage activation status reflect the view of macrophages as effector cells for antimicrobial functions (M1—classical activation) or tissue reparative, antihelminth functions (M2—alternative activation). Although widely acknowledged as an oversimplification, the fundamental idea remains;

that acute signals during an immune response, typically T-cell derived but also pathogen and damage-associated molecular patterns, act to instruct macrophages to perform key host-protective functions. Critically, these activation signals differ from the tissue conditioning factors that determine cell identity described above. Activation is acute, and likely to be dominant over identity. Nonetheless, cell identity will determine the capacity of the cell to respond to specific activation signals, not least due to receptor expression that may be altered by tissue-specific conditioning signals. For example, recently recruited macrophages respond differently to IL-4 than TRMs [83] so prior programming is important in the quality of the macrophage response to stimuli. In addition, unique tissue conditions also control activation response. For example, lung surfactants, the unique metabolic environment and epithelial cell-derived signals, determine the capacity of airway macrophages to respond to IL-4 [84, 85], and proinflammatory signals [86]. Thus, a central question for the field is: **How does the interaction of tissue-specific cell identity with activation signals translate to function?**

### Innate memory

There is increasing evidence that prior history of inflammation and infection can significantly alter the progression of future disease [87], and that macrophages play a central role in this altered state [26, 75, 88, 89]. Coupled with this, the relationship of cell identity to activation state, discussed above, has raised the question **to what degree does prior history determine the macrophage response to future stimuli?** This question lies at the heart of the extensive debate on innate memory or “training,” a concept that was largely developed out of the myeloid cell field [90, 91]. Divangahi, Netea and colleagues [92] recently distinguished trained immunity from other forms of adaptation, by the requirement for the immune activation status to return to basal levels after removal of the stimulus, while epigenetic modification of chromatin and DNA persist. Although this definition may work on an individual cell level, on a tissue level this is challenging, as there are few circumstances in which a tissue challenged by infection or injury returns fully to baseline. For example, extracellular matrix composition can be substantially altered with consequences for the subsequent immune response [93, 94] and inflammation can also lead to long-term alteration of the composition of immune cells within a tissue [87]. Equally, epigenetic modifications underpinning altered tissue responses may reside in other resident cells such as epithelial stem cells [95] and NK cells [96, 97]. These complexities mean the term “training” as currently defined may have limited utility. Semantics aside, the

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Influenza infection can lead to the protracted increase in total alveolar TRM numbers due to persistence of established cells and endurance of monocyte-derived macrophages (iii). In this setting, recruited cells retain an epigenetic and transcriptional legacy of their monocyte origin and provide enhanced resistance to secondary bacterial challenge through elevated IL-6 production [26]. However, whether retention of the monocyte-signature arises from competition-inhibited maturation remains unclear. (D) We propose a model whereby the effect of differentiation of monocytes in the presence of enduring resident TRM is tissue-specific, with recently recruited cells acquiring an inflammatory function between that of established TRM and monocytes.



major outstanding question is **to what extent do macrophage-intrinsic epigenetic modifications give rise to innate memory in tissues?** or does reprogramming of other tissue cells or structures dictate divergent responses of macrophages to future activation? This review is written in the midst of the Covid-19 pandemic and understanding the consequences to lung function of prior viral infection [87] is relevant to the millions who have been infected.

**What is the in vivo evidence that there is cell-intrinsic reprogramming of macrophage responses to new stimuli?** Transfer experiments provide growing evidence that TRMs retain the imprint of prior inflammation. For example, approximately 1 month after resolution of infection with a lung migrating nematode, alveolar macrophages can confer heightened protection to naïve mice [98]. Influenza infection leads to a long-term state of immunosuppression and increased susceptibility to bacterial pneumonia, which can be conferred to naïve mice by transfer of alveolar macrophages and is reversed by replacement of this compartment from the BM [75]. Although these studies suggest long-term cell-intrinsic functional changes to TRMs, we do not know: **where or when are TRMs epigenetically altered during an inflammatory response?** More recently, LPS injection has been shown to affect the severity of subsequent neuropathologies with epigenetic alterations in microglial enhancer repertoires strongly implicated [99]. Pulmonary adenovirus infection results in enhanced resistance to *Streptococcal pneumonia* for at least 4 months, which can be conferred to naïve mice by transfer of the resident alveolar macrophages [88]. These studies provide strong evidence that cell-intrinsic alterations to established TRM can occur in the inflamed tissue itself. However, during an inflammatory response, TRMs can be replaced by BM-derived cells raising the question: **Do key events in education of monocyte-derived macrophages occur following their entry into the tissue or up-stream in the BM?** In a seminal study, pulmonary gammaherpes virus infection led to long-term protection against development of lung allergy due to the replacement of resident alveolar macrophages by long-lived monocyte-derived cells [27]. Only BM-derived TRMs recruited during active infection adopted the immunosuppressive phenotype. However, consistent with some programming at the BM stage, circulating monocytes exhibited elevated MHCII expression and IL-10 production during the acute phase of infection [27]. Very strong evidence that infection can programme hematopoietic stem cells to generate myeloid cells with altered function comes from a study showing that BCG infection bestows an enhanced capacity to kill *Mycobacterium tuberculosis* upon BM-derived macrophages for at least 4–5 months postinfection [100]. This effect was associated with altered histone acetylation and methylation status of BM-derived macrophages and inherited by newly generated monocytes following bone marrow transplant. Taken together, these studies argue for functionally important cell-intrinsic changes to macrophages that alter their innate response to secondary challenge. Yet to more fully understand the cause and consequence of this reprogramming, we need to ask: **How does the effect of the primary stimulus (e.g. strength, quality) on subsequent responses differ depending on the stage of macrophage differ-**

**entiation?** (i.e. BM precursors/monocytes vs recruited inflammatory macrophage vs established TRM).

In our effort to understand the innate mechanisms involved in altered macrophage function, we are in danger of forgetting the potent role of the adaptive immune response, raising the question: **How does adaptive immunity contribute to innate memory?** It remains unclear whether macrophage education by non-adaptive immune mechanisms plays a major physiological role in the context of a fully functioning adaptive immune response. Indeed, the adaptive immune response itself may be the initial “training” event. For example, T-cell-derived IFN- $\gamma$  may imprint long-term epigenetic changes on myeloid cells [88]. Licensing of innate memory by adaptive immunity in infection settings makes certain sense not only given its dominant role in dictating macrophage activation states but also the stringent checkpoints applied to adaptive immunity. **Does adaptive immunity regulate the extent of innate macrophage training, perhaps via T-regulatory cells?** Once innate memory is established, it is easy to imagine that memory T cells will be the dominant determinant of macrophage activation status during most secondary challenges. Nonetheless, epigenetic alterations due to prior exposure will fine-tune macrophage response to these activation signals.

## What is plasticity?

Plasticity is often hailed as a trademark of macrophages and yet what this actually means, and more important its functional consequences, are often unclear. In their original discussion of plasticity, Stout and Suttles [101] proposed that the macrophage lineage displays extreme phenotypic heterogeneity because of their ability to functionally adapt to *changes* in their tissue microenvironment. In this model, macrophage identity or activation state is purely the product of environmental signals at a particular point in time. The circumstances that lead to cell identity (ontogeny, time, tissue conditioning factors) are distinct from more acute factors (T-cell derived cytokines, DAMPS, and microbial products) that drive the current activation state. As discussed above, macrophage function and identity can change due to prior inflammatory events. Inherent in our realization of the effects of ontogeny, time-of-residency and prior inflammatory exposure is to acknowledge that macrophage plasticity is not unrestricted but limited by cell life history. Indeed, one of the challenges of studying plasticity is the term is now used to describe fundamentally different processes.

## Plasticity or “reversibility” of cell identity

As discussed above, TRM identity is dependent on continual exposure to local conditioning signals. For example, while retinoic acid is a major signal controlling the transcriptional identity of serous cavity macrophages, this programme is largely reversible by vitamin A deficiency [59] and the retinoic acid-driven gene programme is lost following in vitro culture [60, 102]. Similarly, microglia removed from the brain lose their transcriptional

programme but can fully reacquire it upon re-engraftment, suggesting the brain is replete with necessary factors to sustain and reinduce homeostatic microglial gene expression in a cell already preconditioned [66]. TRMs in multiple tissues are programmed not to mount an inflammatory response to nucleic acid on clearance of dying cells, but this tolerance is rapidly lost on culture in vitro [103]. So while it is clear that a resident macrophage loses identity when it leaves the tissue niche, **to what degree can resident cell identity be reprogrammed to a new environment?** This question is not just academic. Resident serous cavity macrophages have been reported to migrate into injured organs [104], and the niche itself may change with inflammation/fibrosis.

However, the finding that serous cavity macrophages move into new tissues has been challenged [105], and experimental transfer studies suggest TRMs do not survive well in all ectopic environments. For example, while a small number of peritoneal macrophages can establish when transferred to a macrophage-deficient lung, they fail to repopulate the empty lung, in contrast to the proficient ability of fetal liver or BM-derived monocytes, or yolk-sac macrophages [54]. A similar study found peritoneal macrophages acquired only 70% of the transcriptional identity of alveolar macrophages [106]. These data suggest that the plasticity of mature tissue-resident cells is limited in comparison to monocytes, but why is not known. They may lack the ability to migrate to all available niches in the lung, although in this study, alveolar macrophages were capable of engrafting, suggesting that the peritoneal macrophages have lost the ability to respond to new tissue-specific signals. Indeed, the fundamental question is **What determines the limits of identity plasticity?** The answer likely lies, at least in part, in the consequences of time spent in the tissues, and the epigenetic changes and limitations that may impose. When peritoneal macrophages move into endometrial lesions they lose expression of GATA6 [107] as they do in culture. In contrast, other peritoneal TRM genes that are RA-independent, such as CXCL13, are not lost in culture [61, 102]. Greater understanding the epigenetic regulation of these genes during inflammation or in ectopic environments is needed [102]. Long-term resident cells may have more limited plasticity because they no longer express key receptors or because monocyte enhancers are no longer poised to respond [106]. Once again, these are not just academic questions, since established peritoneal macrophages are less effective at preventing endometrial lesion growth than recently monocyte-derived resident macrophages [107].

### Plasticity or “reversibility” of activation state

References to macrophage plasticity in the literature are frequently based not on cell identity as discussed above, but on the ability of macrophages to move reversibly on a spectrum (or a wheel) between M1/M2 activation states [3, 108]. The potential for reprogramming is particularly relevant in the context of infection, where consecutive infections with diverse pathogens and the requirement to repair infection damage are major evolutionary drivers. Our understanding of plasticity, however, has been heavily

drawn from in-vitro or ex-vivo studies in which cells are removed and repolarized [101, 109]. While in-vivo studies strongly implicate the ability of macrophages to alter their activation state, studies that demonstrate plasticity on a single-cell level are rare. In a coinfection study, peritoneal TRMs activated and expanded by IL-4 (M(IL-4)) during helminth infection, reduce type 2 markers and produce iNOS in response to subsequent *Salmonella* infection [89]. Using RELM $\alpha$  as marker for M(IL-4), individual RELM $\alpha$  cells are equal or better able to produce iNOS than RELM $\alpha$ -cells in response to bacteria. Another finding was that activation plasticity was not, as often assumed, bidirectional. While microbial signals could reprogramme a strongly M(IL-4) polarized macrophage, the reverse was not true [89], consistent with a report that suppression of mitochondrial function in M1 cells prevents M(IL-4) repolarization [110]. So not surprisingly, there are constraints on plasticity but **What determines the limits of activation plasticity?** While these constraints may be largely epigenetic, cellular location can also influence the ability of a macrophage to be reprogrammed. Dermal macrophages retain a full M(IL-4) phenotype in the face of a potent Th1-tissue environment caused by *Leishmania major* infection and appear protected from the Th1-stimuli by IL-4 producing eosinophils [111]. It remains unclear whether these cells are rendered unresponsive to proinflammatory/Th1 stimuli or simply spatially detached or physically protected from these signals. Indeed, one of the greatest limitations to understanding the extent of reversibility to activate in vivo is our inability to definitively measure which stimuli individual cells have been exposed to both during initial activation and subsequent activation. Perhaps, a more fundamental question arises: **How important is activation plasticity at the single-cell level?** Despite clear evidence of TRM plasticity from M2 to M1 as described above, monocyte influx is the dominant protective response to *Salmonella* infection, regardless of TRM numbers or phenotype [89]. Indeed, to understand the contribution of macrophages to specific disease outcomes, the practical question remains **What are the distinct host-protective functions of TRMs vs. newly recruited cells?** There is certainly no simple paradigm that answers this question. Incoming monocyte-derived cells can be either more proinflammatory [26, 89] or more immune suppressive [73, 112] than the existing TRMs. These differences may be linked to the cells proliferative state [50], innate memory as discussed above, and competition between these populations (see Fig. 3B and D)

### Conclusions

Irrespective of all the question we have highlighted, understanding the role macrophages in disease remains of primary importance and we want to pay homage to the plethora of articles defining new functions of macrophages in different contexts and different tissues. While we cannot do justice to the large number of new articles, it is important to highlight that we are now beginning to answer one of the most pressing questions in the field: **What are the functional consequences of macrophage life history?** Whether the cell is blood derived, short-term resident, or

long-term resident matters because therapeutic approaches can be targeted. It is critical to remember that the vast majority of work cited in this review was performed in C57BL/6 mice because of the utility of this strain. However, the focus on a single genetic background means gene by environment interactions are largely missed in our current approaches. In addition to expanding our animal studies to include a greater range of natural conditions, more experimental work in humans [113, 114], where ethically possible, is needed. Fortunately, the field is currently benefitting enormously from single-cell technologies, which are dramatically enhancing our ability to identify myeloid populations that are conserved between mice and humans [19, 115–117].

**Acknowledgments:** We acknowledge the support of the Medical Research Council UK (MR/K01207X/2 to JEA and MR/L008076/1 to SJ) and the Wellcome Trust (106898/A/15/Z to JEA). We thank C. Finlay, J. Ajendra, and P. Papotto for constructive review of the manuscript. Figures were created with Biorender.com

**Conflict of interest:** Authors have no conflict of interest.

## Literature Cited

- Doyle, A. G., Herbein, G., Montaner, L. J., Minty, A. J., Caput, D., Ferrara, P., Gordon, S., Interleukin-13 alters the activation state of murine macrophages in vitro: comparison with interleukin-4 and interferon-gamma. *Eur. J. Immunol.* 1994. 24: 1441–1445.
- Wynn, T. A., Chawla, A., Pollard, J. W., Macrophage biology in development, homeostasis and disease. *Nature* 2013. 496: 445–455.
- Mosser, D. M., Edwards, J. P., Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 2008. 8: 958–969.
- Sieweke, M. H., Allen, J. E., Beyond stem cells: self-renewal of differentiated macrophages. *Science* 2013. 342: 1242974.
- Blériot, C., Chakarov, S., Ginhoux, F., Determinants of resident tissue macrophage identity and function. *Immunity* 2020. 52: 957–970.
- Guilliams, M., Thierry, G. R., Bonnardel, J., Bajenoff, M., Establishment and maintenance of the macrophage niche. *Immunity* 2020. 52: 434–451.
- Jenkins, S. J., Ruckerl, D., Thomas, G. D., Hewitson, J. P., Duncan, S., Brombacher, F., Maizels, R. M. et al., IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. *J. Exp. Med.* 2013. 210: 2477–2491.
- Jenkins, S. J., Ruckerl, D., Cook, P. C., Jones, L. H., Finkelman, F. D., van Rooijen, N., MacDonald, A. S. et al., Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* 2011. 332: 1284–1288.
- Liu, Z., Gu, Y., Chakarov, S., Blériot, C., Kwok, I., Chen, X., Shin, A. et al., Fate mapping via Ms4a3-expression history traces monocyte-derived cells. *Cell* 2019. 178: 1509–1525.e19.
- Bain, C. C., Hawley, C. A., Garner, H., Scott, C. L., Schridde, A., Steers, N. J., Mack, M. et al., Long-lived self-renewing bone marrow-derived macrophages displace embryo-derived cells to inhabit adult serous cavities. *Nat. Commun.* 2016. 7: ncomms11852.
- Zhou, X., Franklin, R. A., Adler, M., Jacox, J. B., Bailis, W., Shyer, J. A., Flavell, R. A. et al., Circuit design features of a stable two-cell system. *Cell* 2018. 172: 744–757.e17.
- Jenkins, S. J., Hume, D. A., Homeostasis in the mononuclear phagocyte system. *Trends Immunol.* 2014. 35: 358–367.
- Bonnardel, J., T'Jonck, W., Gaublumme, D., Browaeys, R., Scott, C. L., Martens, L., Vanneste, B. et al., Stellate cells, hepatocytes, and endothelial cells imprint the kupffer cell identity on monocytes colonizing the liver macrophage niche. *Immunity* 2019. 51: 638–654.e9.
- Westphalen, K., Gusarova, G. A., Islam, M. N., Subramanian, M., Cohen, T. S., Prince, A. S., Bhattacharya, J., Sessile alveolar macrophages communicate with alveolar epithelium to modulate immunity. *Nature* 2014. 506: 503–506.
- Uderhardt, S., Martins, A. J., Tsang, J. S., Lämmermann, T., Germain, R. N., Resident macrophages cloak tissue microlesions to prevent neutrophil-driven inflammatory damage. *Cell* 2019. 177: 541–555.
- Baranska, A., Shawket, A., Jouve, M., Baratin, M., Malosse, C., Voluzan, O., Vu Manh, T.-P. et al., Unveiling skin macrophage dynamics explains both tattoo persistence and strenuous removal. *J. Exp. Med.* 2018. 215: 1115–1133.
- Pridans, C., Irvine, K. M., Davis, G. M., Lefevre, L., Bush, S. J., Hume, D. A., Transcriptomic analysis of rat macrophages. *Front. Immunol.* 2021. 11: 594594.
- Tang, J., Frey, J. M., Wilson, C. L., Moncada-Pazos, A., Levet, C., Freeman, M., Rosenfeld, M. E. et al., Neutrophil and macrophage cell surface colony-stimulating factor 1 shed by ADAM17 drives mouse macrophage proliferation in acute and chronic inflammation. *Mol. Cell. Biol.* 2018. 38: e00103–18.
- Joshi, N., Watanabe, S., Verma, R., Jablonski, R. P., Chen, C.-I., Cheresch, P., Markov, N. S. et al., A spatially restricted fibrotic niche in pulmonary fibrosis is sustained by M-CSF/M-CSFR signalling in monocyte-derived alveolar macrophages. *Eur. Respir. J.* 2020. 55: 1900646.
- Blériot, C., Dupuis, T., Jouvion, G., Eberl, G., Disson, O., Lecuit, M., Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. *Immunity* 2015. 42: 145–158.
- Tran, S., Baba, I., Poupel, L., Dussaud, S., Moreau, M., Gélinau, A., Marcelin, G. et al., Impaired kupffer cell self-renewal alters the liver response to lipid overload during non-alcoholic steatohepatitis. *Immunity* 2020. 53: 627–640.e5.
- Ferrer, I. R., West, H. C., Henderson, S., Ushakov, D. S., Santos, E., Sousa, P., Strid, J. et al., A wave of monocytes is recruited to replenish the long-term Langerhans cell network after immune injury. *Sci. Immunol.* 2019. 4: eaax8704.
- Yona, S., Kim, K.-W., Wolf, Y., Mildner, A., Varol, D., Breker, M., Strauss-Ayali, D. et al., Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 2013. 38: 79–91.
- Louwe, P. A., Badiola Gomez, L., Webster, H., Perona-Wright, G., Bain, C. C., Forbes, S. J., Jenkins, S. J., Recruited macrophages that colonize the post-inflammatory peritoneal niche convert into functionally divergent resident cells. *Nat. Commun.* 2021. 12: 1770.
- Misharin, A. V., Morales-Nebreda, L., Reyfman, P. A., Cuda, C. M., Walter, J. M., McQuattie-Pimentel, A. C., Chen, C.-I. et al., Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. *J. Exp. Med.* 2017. 214: 2387–2404.
- Aegerter, H., Kulikauskaite, J., Crotta, S., Patel, H., Kelly, G., Hessel, E. M., Mack, M. et al., Influenza-induced monocyte-derived alveolar

- macrophages confer prolonged antibacterial protection. *Nat. Immunol.* 2020. 21: 145–157.
- 27 Machiels, B., Dourcy, M., Xiao, X., Javaux, J., Mesnil, C., Sabatel, C., Desmecht, D. et al., A gamma herpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes. *Nat. Immunol.* 2017. 18: 1310–1320.
  - 28 Rua, R., Lee, J. Y., Silva, A. B., Swafford, I. S., Maric, D., Johnson, K. R., McGavern, D. B., Infection drives meningeal engraftment by inflammatory monocytes that impairs CNS immunity. *Nat. Immunol.* 2019. 20: 407–419.
  - 29 Scott, C. L., Zheng, F., De Baetselier, P., Martens, L., Saeys, Y., De Prijck, S., Lippens, S. et al., Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nat. Commun.* 2016. 7: 10321.
  - 30 Davies, L. C., Rosas, M., Jenkins, S. J., Liao, C.-T., Scurr, M. J., Brombacher, F., Fraser, D. J. et al., Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. *Nat. Commun.* 2013. 4: 1886.
  - 31 Davies, L. C., Rosas, M., Smith, P. J., Fraser, D. J., Jones, S. A., Taylor, P. R., A quantifiable proliferative burst of tissue macrophages restores homeostatic macrophage populations after acute inflammation. *Eur. J. Immunol.* 2011. 41: 2155–2164.
  - 32 Hawley, C. A., Rojo, R., Raper, A., Sauter, K. A., Lisowski, Z. M., Grabert, K., Bain, C. C. et al., Csf1r-mApple transgene expression and ligand binding in vivo reveal dynamics of CSF1R expression within the mononuclear phagocyte system. *J. Immunol.* 2018. 200: 2209–2223.
  - 33 Tagliani, E., Shi, C., Nancy, P., Tay, C.-S., Pamer, E. G., Erlebacher, A., Coordinate regulation of tissue macrophage and dendritic cell population dynamics by CSF-1. *J. Exp. Med.* 2011. 208: 1901–1916.
  - 34 Tymoszek, P., Evens, H., Marzola, V., Wachowicz, K., Wasmer, M.-H., Datta, S., Müller-Holzner, E. et al., In situ proliferation contributes to accumulation of tumor-associated macrophages in spontaneous mammary tumors. *Eur. J. Immunol.* 2014. 44: 2247–2262.
  - 35 Soncin, I., Sheng, J., Chen, Q., Foo, S., Duan, K., Lum, J., Poidinger, M. et al., The tumour microenvironment creates a niche for the self-renewal of tumour-promoting macrophages in colon adenoma. *Nat. Commun.* 2018. 9: 582.
  - 36 Tamoutounour, S., Guillemins, M., Montanana Sanchis, F., Liu, H., Terhorst, D., Malosse, C., Pollet, E. et al., Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* 2013. 39: 925–938.
  - 37 Bain, C. C., Bravo-Blas, A., Scott, C. L., Perdiguero, E. G., Geissmann, F., Henri, S., Malissen, B. et al., Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat. Immunol.* 2014. 15: 929–937.
  - 38 Ghigo, C., Mondor, I., Jorquera, A., Nowak, J., Wienert, S., Zahner, S. P., Clausen, B. E. et al., Multicolor fate mapping of Langerhans cell homeostasis. *J. Exp. Med.* 2013. 210: 1657–1664.
  - 39 Tay, T. L., Mai, D., Dautzenberg, J., Fernández-Klett, F., Lin, G., Sagar, Datta M et al., A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat. Neurosci.* 2017. 20: 793–803.
  - 40 Réu, P., Khosravi, A., Bernard, S., Mold, J. E., Salehpour, M., Alkass, K., Perl, S. et al., The lifespan and turnover of microglia in the human brain. *Cell Rep.* 2017. 20: 779–784.
  - 41 Askew, K., Li, K., Olmos-Alonso, A., Garcia-Moreno, F., Liang, Y., Richardson, P., Tipton, T. et al., Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. *Cell Rep.* 2017. 18: 391–405.
  - 42 Amano, S. U., Cohen, J. L., Vangala, P., Tencerova, M., Nicoloso, S. M., Yawe, J. C., Shen, Y. et al., Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. *Cell Metab.* 2014. 19: 162–171.
  - 43 Milner, J. D., Orekov, T., Ward, J. M., Cheng, L., Torres-Velez, F., Junttila, I., Sun, G. et al., Sustained IL-4 exposure leads to a novel pathway for hemophagocytosis, inflammation, and tissue macrophage accumulation. *Blood* 2010. 116: 2476–2483.
  - 44 Rückerl, D., Allen, J. E., Macrophage proliferation, provenance, and plasticity in macroparasite infection. *Immunol. Rev.* 2014. 262: 113–133.
  - 45 Knipper, J. A., Willenborg, S., Brinckmann, J., Bloch, W., Maaß, T., Wagener, R., Krieg, T. et al., Interleukin-4 receptor  $\alpha$  signaling in myeloid cells controls collagen fibril assembly in skin repair. *Immunity* 2015. 43: 803–816.
  - 46 Van Gassen, N., Van Overmeire, E., Leuckx, G., Heremans, Y., De Groef, S., Cai, Y., Elkrim, Y. et al., Macrophage dynamics are regulated by local macrophage proliferation and monocyte recruitment in injured pancreas. *Eur. J. Immunol.* 2015. 45: 1482–1493.
  - 47 Robbins, C. S., Hilgendorf, I., Weber, G. F., Theurl, I., Iwamoto, Y., Figueiredo, J.-L., Gorbato, R. et al., Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat. Med.* 2013. 19: 1166–1172.
  - 48 Zhu, Y., Herndon, J. M., Sojka, D. K., Kim, K.-W., Knolhoff, B. L., Zuo, C., Cullinan, D. R. et al., Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity* 2017. 47: 323–338.e6.
  - 49 Sanin, D. E., Matsushita, M., Klein Geltink, R. I., Grzes, K. M., van Teijlingen Bakker, N., Corrado, M., Kabat, A. M. et al., Mitochondrial membrane potential regulates nuclear gene expression in macrophages exposed to prostaglandin E2. *Immunity* 2018. 49: 1021–1033.e6.
  - 50 Zhu, B., Wu, Y., Huang, S., Zhang, R., Son, Y. M., Li, C., Cheon, I. S. et al., Uncoupling of macrophage inflammation from self-renewal modulates host recovery from respiratory viral infection. *Immunity* 2021. 54: 1–19.
  - 51 Soucie, E. L., Weng, Z., Geirsdóttir, L., Molawi, K., Maurizio, J., Fenouil, R., Mossadegh-Keller, N. et al., Lineage-specific enhancers activate self-renewal genes in macrophages and embryonic stem cells. *Science* 2016. 351: aad5510.
  - 52 Lawrence, T., Natoli, G., Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat. Rev. Immunol.* 2011. 11: 750–761.
  - 53 Gibbings, S. L., Goyal, R., Desch, A. N., Leach, S. M., Prabagar, M., Atif, S. M., Bratton, D. L. et al., Transcriptome analysis highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. *Blood* 2015. 126: 1357–1366.
  - 54 van de Laar, L., Saelens, W., De Prijck, S., Martens, L., Scott, C. L., Van Isterdael, G., Hoffmann, E. et al., Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. *Immunity* 2016. 44: 755–768.
  - 55 Beattie, L., Sawtell, A., Mann, J., Frame, T. C. M., Teal, B., de Labastida Rivera, F., Brown, N. et al., Bone marrow-derived and resident liver macrophages display unique transcriptomic signatures but similar biological functions. *J. Hepatol.* 2016. 65: 758–768.
  - 56 David, B. A., Rezende, R. M., Antunes, M. M., Santos, M. M., Freitas Lopes, M. A., Diniz, A. B., Sousa Pereira, R. V. et al., Combination of mass cytometry and imaging analysis reveals origin, location, and functional repopulation of liver myeloid cells in mice. *Gastroenterology* 2016. 151: 1176–1191.



- 57 Suzuki, T., Arumugam, P., Sakagami, T., Lachmann, N., Chalk, C., Sallese, A., Abe, S. et al., Pulmonary macrophage transplantation therapy. *Nature* 2014. 514: 450–454.
- 58 Cronk, J. C., Derecki, N. C., Ji, E., Xu, Y., Lampano, A. E., Smirnov, I., Baker, W. et al., Methyl-CpG binding protein 2 regulates microglia and macrophage gene expression in response to inflammatory stimuli. *Immunity* 2015. 42: 679–691.
- 59 Okabe, Y., Medzhitov, R., Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell* 2014. 157: 832–844.
- 60 Rosas, M., Davies, L. C., Giles, P. J., Liao, C.-T., Kharfan, B., Stone, T. C., O'Donnell, V. B. et al., The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. *Science* 2014. 344: 645–648.
- 61 Buechler, M. B., Kim, K.-W., Onufer, E. J., Williams, J. W., Little, C. C., Dominguez, C. X., Li, Q. et al., A stromal niche defined by expression of the transcription factor WT1 mediates programming and homeostasis of cavity-resident macrophages. *Immunity* 2019. 51: 1–12.
- 62 Scott, C. L., T'Jonck, W., Martens, L., Todorov, H., Sichien, D., Soen, B., Bonnardel, J. et al., The transcription factor ZEB2 is required to maintain the tissue-specific identities of macrophages. *Immunity* 2018. 49: 312–325.e5.
- 63 Mass, E., Ballesteros, I., Farlik, M., Halbritter, F., Günther, P., Crozet, L., Jacome-Galarza, C. E. et al., Specification of tissue-resident macrophages during organogenesis. *Science* 2016. 353: aaf4238.
- 64 Sakai, M., Troutman, T. D., Seidman, J. S., Ouyang, Z., Spann, N. J., Abe, Y., Ego, K. M. et al., Liver-derived signals sequentially reprogram myeloid enhancers to initiate and maintain Kupffer cell identity. *Immunity* 2019. 51: 655–670.
- 65 Shemer, A., Grozovski, J., Tay, T. L., Tao, J., Volaski, A., Süß, P., Ardura-Fabregat, A. et al., Engrafted parenchymal brain macrophages differ from microglia in transcriptome, chromatin landscape and response to challenge. *Nat. Commun.* 2018. 9: 5206.
- 66 Bennett, F. C., Bennett, M. L., Yaqoob, F., Mulinyawe, S. B., Grant, G. A., Hayden Gephart, M., Plowey, E. D. et al., A combination of ontogeny and CNS environment establishes microglial identity. *Neuron* 2018. 98: 1170–1183.
- 67 Lund, H., Pieber, M., Parsa, R., Han, J., Grommisch, D., Ewing, E., Kular, L. et al., Competitive repopulation of an empty microglial niche yields functionally distinct subsets of microglia-like cells. *Nat. Commun.* 2018. 9: 4845.
- 68 Cronk, J. C., Filiano, A. J., Louveau, A., Marin, I., Marsh, R., Ji, E., Goldman, D. H. et al., Peripherally derived macrophages can engraft the brain independent of irradiation and maintain an identity distinct from microglia. *J. Exp. Med.* 2018. 215: 1627–1647.
- 69 Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F. et al., Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010. 330: 841–845.
- 70 Matcovitch-Natan, O., Winter, D. R., Giladi, A., Vargas Aguilar, S., Spinrad, A., Sarrazin, S., Ben-Yehuda, H. et al., Microglia development follows a stepwise program to regulate brain homeostasis. *Science* 2016. 353: aad8670.
- 71 Bain, C. C., Gibson, D. A., Steers, N. J., Boufe, K., Louwe, P. A., Doherty, C., González-Huici, V. et al., Rate of replenishment and microenvironment contribute to the sexually dimorphic phenotype and function of peritoneal macrophages. *Sci. Immunol.* 2020. 5: eabc4466.
- 72 Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., Liu, W. et al., Metabolic regulation of gene expression by histone lactylation. *Nature* 2019. 574: 575–580.
- 73 Campbell, S. M., Knipper, J. A., Ruckerl, D., Finlay, C. M., Logan, N., Minutti, C. M., Mack, M. et al., Myeloid cell recruitment versus local proliferation differentiates susceptibility from resistance to filarial infection. *Elife* 2018. 7: e30947.
- 74 Zhang, N., Czepielewski, R. S., Jarjour, N. N., Erlich, E. C., Esaulova, E., Saunders, B. T., Grover, S. P. et al., Expression of factor V by resident macrophages boosts host defense in the peritoneal cavity. *J. Exp. Med.* 2019. 216: 1291–1300.
- 75 Didierlaurent, A., Goulding, J., Patel, S., Snelgrove, R., Low, L., Bebiën, M., Lawrence, T. et al., Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. *J. Exp. Med.* 2008. 205: 323–329.
- 76 Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H. et al., Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 2015. 18: 965–977.
- 77 Scott, N. A., Mann, E. R., Regulation of mononuclear phagocyte function by the microbiota at mucosal sites. *Immunology* 2020. 159: 26–38.
- 78 McDonald, B., Zucoloto, A. Z., Yu, I.-L., Burkhard, R., Brown, K., Geuking, M. B., McCoy, K. D., Programming of an intravascular immune firewall by the gut microbiota protects against pathogen dissemination during infection. *Cell Host Microbe*. 2020. 28: 660–668.e4.
- 79 Gola, A., Dorington, M. G., Speranza, E., Sala, C., Shih, R. M., Radtke, A. J., Wong, H. S. et al., Commensal-driven immune zonation of the liver promotes host defence. *Nature* 2021. 589: 131–136.
- 80 Villa, A., Gelosa, P., Castiglioni, L., Cimino, M., Rizzi, N., Pepe, G., Lolli, F. et al., Sex-specific features of microglia from adult mice. *Cell Rep.* 2018. 23: 3501–3511.
- 81 Markle, J. G. M., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolfe-Kampczyk, U., von Bergen, M. et al., Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 2013. 339: 1084–1088.
- 82 Thion, M. S., Low, D., Silvin, A., Chen, J., Grisel, P., Schulte-Schrepping, J., Blecher, R. et al., Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell* 2018. 172: 500–516.
- 83 Gundra, U. M., Girgis, N. M., Ruckerl, D., Jenkins, S., Ward, L. N., Kurtz, Z. D., Wiens, K. E. et al., Alternatively activated macrophages derived from monocytes and tissue macrophages are phenotypically and functionally distinct. *Blood* 2014. 123: e110–122.
- 84 Minutti, C. M., Jackson-Jones, L. H., García-Fojeda, B., Knipper, J. A., Sutherland, T. E., Logan, N., Ringqvist, E. et al., Local amplifiers of IL-4Ra-mediated macrophage activation promote repair in lung and liver. *Science* 2017. 356: 1076–1080.
- 85 Svedberg, F. R., Brown, S. L., Krauss, M. Z., Campbell, L., Sharpe, C., Clausen, M., Howell, G. J. et al., The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. *Nat. Immunol.* 2019. 20: 571–580.
- 86 Kaur, M., Bell, T., Salek-Ardakani, S., Hussell, T., Macrophage adaptation in airway inflammatory resolution. *Eur. Respir. Rev.* 2015. 24: 510–515.
- 87 Goulding, J., Snelgrove, R., Saldana, J., Didierlaurent, A., Cavanagh, M., Gwyer, E., Wales, J. et al., Respiratory infections: do we ever recover? *Proc. Am. Thorac. Soc.* 2007. 4: 618–625.
- 88 Yao, Y., Jeyanathan, M., Haddadi, S., Barra, N. G., Vaseghi-Shanjani, M., Damjanovic, D., Lai, R. et al., Induction of autonomous memory alveolar macrophages requires T cell help and is critical to trained immunity. *Cell* 2018. 175: 1634–1650.e17.
- 89 Rückerl, D., Campbell, S. M., Duncan, S., Sutherland, T. E., Jenkins, S. J., Hewitson, J. P., Barr, T. A. et al., Macrophage origin limits functional plasticity in helminth-bacterial co-infection. *PLoS Pathog.* 2017. 13: e1006233.

- 90 Bowdish, D. M. E., Loffredo, M. S., Mukhopadhyay, S., Mantovani, A., Gordon, S., Macrophage receptors implicated in the “adaptive” form of innate immunity. *Microbes Infect.* 2007. **9**: 1680–1687.
- 91 Netea, M. G., Quintin, J., van der Meer, J. W. M., Trained immunity: a memory for innate host defense. *Cell Host Microbe.* 2011. **9**: 355–361.
- 92 Divangahi, M., Aaby, P., Khader, S. A., Barreiro, L. B., Bekkering, S., Chavakis, T., van Crevel, R. et al., Trained immunity, tolerance, priming and differentiation: distinct immunological processes. *Nat. Immunol.* 2021. **22**: 2–6.
- 93 Boyd, D. F., Allen, E. K., Randolph, A. G., Guo, X.-Z. J., Weng, Y., Sanders, C. J., Bajracharya, R. et al., Exuberant fibroblast activity compromises lung function via ADAMTS4. *Nature* 2020. **587**: 466–471.
- 94 McQuattie-Pimentel, A. C., Ren, Z., Joshi, N., Watanabe, S., Stoeger, T., Chi, M., Lu, Z. et al., The lung microenvironment shapes a dysfunctional response of alveolar macrophages in aging. *J. Clin. Invest.* 2021. **131**: e140299.
- 95 Naik, S., Larsen, S. B., Gomez, N. C., Alaverdyan, K., Sandoel, A., Yuan, S., Polak, L. et al., Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. *Nature* 2017. **550**: 475–480.
- 96 Schlums, H., Cichocki, F., Tesi, B., Theorell, J., Beziat, V., Holmes, T. D., Han, H. et al., Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. *Immunity* 2015. **42**: 443–456.
- 97 Lee, J., Zhang, T., Hwang, I., Kim, A., Nitschke, L., Kim, M., Scott, J. M. et al., Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. *Immunity* 2015. **42**: 431–442.
- 98 Chen, F., Wu, W., Millman, A., Craft, J. F., Chen, E., Patel, N., Boucher, J. L. et al., Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. *Nat. Immunol.* 2014. **15**: 938–946.
- 99 Wendeln, A.-C., Degenhardt, K., Kaurani, L., Gertig, M., Ulas, T., Jain, G., Wagner, J. et al., Innate immune memory in the brain shapes neurological disease hallmarks. *Nature* 2018. **556**: 332–338.
- 100 Kaufmann, E., Sanz, J., Dunn, J. L., Khan, N., Mendonça, L. E., Pacis, A., Tzelepis, F. et al., BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 2018. **172**: 176–190.
- 101 Stout, R. D., Jiang, C., Matta, B., Tietzel, I., Watkins, S. K., Suttles, J., Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J. Immunol.* 2005. **175**: 342–349.
- 102 Gosselin, D., Link, V. M., Romanoski, C. E., Fonseca, G. J., Eichenfield, D. Z., Spann, N. J., Stender, J. D. et al., Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* 2014. **159**: 1327–1340.
- 103 Roberts, A. W., Lee, B. L., Deguine, J., John, S., Shlomchik, M. J., Barton, G. M., Tissue-resident macrophages are locally programmed for silent clearance of apoptotic cells. *Immunity* 2017. **47**: 913–927.
- 104 Wang, J., Kubes, P., A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair. *Cell* 2016. **165**: 668–678.
- 105 Jin, H., Liu, K., Tang, J., Huang, X., Wang, H., Zhang, Q., Zhu, H. et al., Genetic fate-mapping reveals surface accumulation but not deep organ invasion of pleural and peritoneal cavity macrophages following injury. *Nat. Commun.* 2021. **12**: 2863.
- 106 Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S. et al., Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* 2014. **159**: 1312–1326.
- 107 Hogg, C., Panir, K., Dhami, P., Rosser, M., Mack, M., Soong, D., Pollard, J. W. et al., Macrophages inhibit and enhance endometriosis depending on their origin. *Proc. Natl. Acad. Sci. U. S. A.* 2021. **118**: e2013776118.
- 108 Sica, A., Mantovani, A., Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* 2012. **122**: 787–795.
- 109 Mylonas, K. J., Nair, M. G., Prieto-Lafuente, L., Paape, D., Allen, J. E., Alternatively activated macrophages elicited by helminth infection can be reprogrammed to enable microbial killing. *J. Immunol.* 2009. **182**: 3084–3094.
- 110 Van den Bossche, J., Baardman, J., Otto, N. A., van der Velden, S., Neele, A. E., van den Berg, S. M., Luque-Martin, R. et al., Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Rep.* 2016. **17**: 684–696.
- 111 Lee, S. H., Chaves, M. M., Kamenyeva, O., Gazzinelli-Guimaraes, P. H., Kang, B., Pessenda, G., Passelli, K. et al., M2-like, dermal macrophages are maintained via IL-4/CCL24-mediated cooperative interaction with eosinophils in cutaneous *Leishmaniasis*. *Sci. Immunol.* 2020. **5**: eaaz4415.
- 112 Liu, M., Tong, Z., Ding, C., Luo, F., Wu, S., Wu, C., Albeituni, S. et al., Transcription factor c-Maf is a checkpoint that programs macrophages in lung cancer. *J. Clin. Invest.* 2020. **130**: 2081–2096.
- 113 Patel, A. A., Zhang, Y., Fullerton, J. N., Boelen, L., Rongvaux, A., Maini, A. A., Bigley, V. et al., The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *J. Exp. Med.* 2017. **214**: 1913–1923.
- 114 Pallett, L. J., Burton, A. R., Amin, O. E., Rodriguez-Tajes, S., Patel, A. A., Zakeri, N., Jeffery-Smith, A. et al., Longevity and replenishment of human liver-resident memory T cells and mononuclear phagocytes. *J. Exp. Med.* 2020. **217**: e20200050.
- 115 Zilionis, R., Engblom, C., Pfirschke, C., Savova, V., Zemmour, D., Saatioglu, H. D., Krishnan, I. et al., Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. *Immunity* 2019. **50**: 1317–1334.
- 116 Byrne, A. J., Powell, J. E., O’Sullivan, B. J., Ogger, P. P., Hoffland, A., Cook, J., Bonner, K. L. et al., Dynamics of human monocytes and airway macrophages during healthy aging and after transplant. *J. Exp. Med.* 2020. **217**: e20191236.
- 117 Ramachandran, P., Dobie, R., Wilson-Kanamori, J. R., Dora, E. F., Henderson, B. E. P., Luu, N. T., Portman, J. R. et al., Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* 2019. **575**: 512–518.
- 118 Jackson-Jones, L. H., Rückerl, D., Svedberg, F., Duncan, S., Maizels, R. M., Sutherland, T. E., Jenkins, S. J. et al., IL-33 delivery induces serous cavity macrophage proliferation independent of interleukin-4 receptor alpha. *Eur. J. Immunol.* 2016. **46**: 2311–2321.
- 119 Stanley, E. R., Chitu, V., CSF-1 receptor signaling in myeloid cells. *Cold Spring Harb. Perspect. Biol.* 2014. **6**: a021857.
- 120 Rückerl, D., Jenkins, S. J., Laqtom, N. N., Gallagher, I. J., Sutherland, T. E., Duncan, S., Buck, A. H. et al., Induction of IL-4Rα-dependent microRNAs identifies PI3K/Akt signaling as essential for IL-4-driven murine macrophage proliferation in vivo. *Blood* 2012. **120**: 2307–2316.
- 121 Bosurgi, L., Cao, Y. G., Cabeza-Cabrerizo, M., Tucci, A., Hughes, L. D., Kong, Y., Weinstein, J. S. et al., Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells. *Science* 2017. **356**: 1072–1076.
- 122 Jarjour, N. N., Schwarzkopf, E. A., Bradstreet, T. R., Shchukina, I., Lin, C.-C., Huang, S. C.-C., Lai, C.-W. et al., Bhlhe40 mediates tissue-specific control of macrophage proliferation in homeostasis and type 2 immunity. *Nat. Immunol.* 2019. **20**: 687–700.

- 123 Gautier, E. L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., Helft, J. et al., Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 2012. **13**: 1118.
- 124 Okabe, Y., Medzhitov, R., Tissue biology perspective on macrophages. *Nat. Immunol.* 2016. **17**: 9–17.
- 125 Newson, J., Motwani, M. P., Kendall, A. C., Nicolaou, A., Muccioli, G. G., Alhouayek, M., Bennett, M. et al., Inflammatory resolution triggers a prolonged phase of immune suppression through COX-1/mPGES-1-derived prostaglandin E2. *Cell Rep.* 2017. **20**: 3162–3175.
- 126 Newson, J., Stables, M., Karra, E., Arce-Vargas, F., Quezada, S., Motwani, M., Mack, M. et al., Resolution of acute inflammation bridges the gap between innate and adaptive immunity. *Blood* 2014. **124**: 1748–1764.

**Abbreviations:** **KC:** Kupffer cells · **TRMs:** tissue-resident macrophages · **WT1:** Wilms' Tumor 1

**Full correspondence:** Dr. Stephen J. Jenkins, University of Edinburgh, Centre for Inflammation Research, Queens Medical Research Institute, Edinburgh, EH16 4TJ, UK or Prof. Judith E. Allen, University of Manchester, Lydia Becker Institute of Immunology & Inflammation, Wellcome Centre for Cell Matrix Research, School of Biological Sciences, Manchester, M13 9PT, UK  
e-mail: stephen.jenkins@ed.ac.uk, judi.allen@manchester.ac.uk

Received: 2/3/2021

Revised: 2/3/2021

Accepted: 8/6/2021

Accepted article online: 9/6/2021